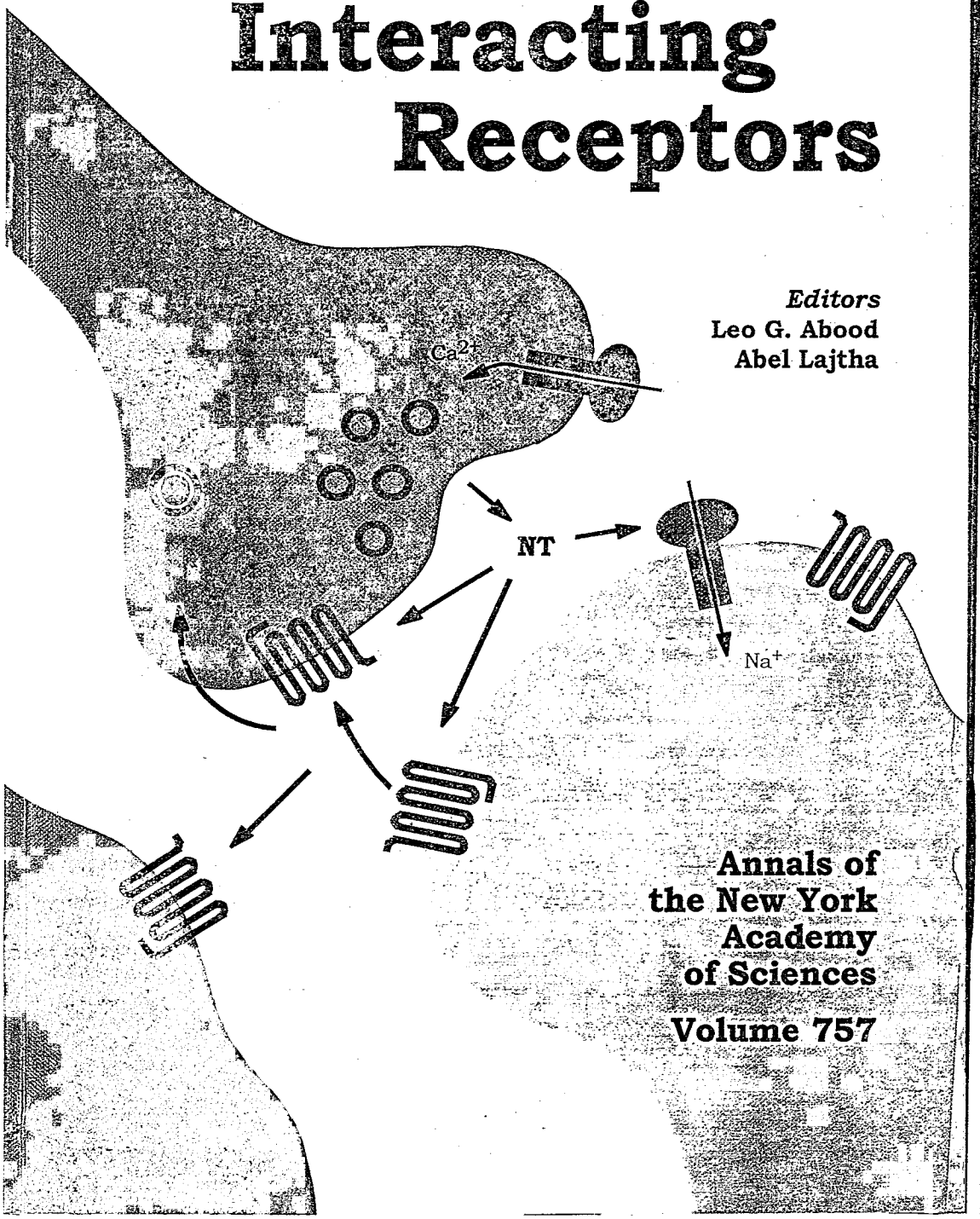
Diversity of Interacting Receptors



Editors
Leo G. Abood
Abel Lajtha

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ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
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DIVERSITY OF INTERACTING RECEPTORS

Edited by Leo G. Abood and Abel Lajtha

The New York Academy of Sciences
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Cover illustration: The cascade of effects caused by the release of a neurotransmitter.

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DIVERSITY OF INTERACTING RECEPTORS[a]

Editors and Conference Organizers
LEO G. ABOOD AND ABEL LAJTHA

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Background and Overview of the Conference

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BACKGROUND

The discovery that the pharmacologic effects of acetylcholine (ACh) and related agents in the autonomic system were mimicked by muscarine or nicotine,[1] followed by the discovery that ACh was released by the frog vagus nerve,[2] introduced the seminal concepts of chemical transmission and the finding that a single chemical transmitter exhibited multiple functions at distinct effector sites. Since that time the number of neurotransmitters and other chemical mediators along with their multiple receptor subtypes has grown at a phenomenal pace. One of the most challenging problems confronting the biologist is the functional implication of multiple forms of receptors and their interactions. The following are examples of the diversity of multiple receptor subtypes, their contributory factors, and functional implications.

- Differences in the time course of neurally elicited responses.
  Example: Fast excitatory postsynaptic potential of nicotinic receptors and slow excitatory postsynaptic potential of muscarinic cholinergic receptors.[3,4]
- Receptors linked to signal transduction systems require structural alterations for specific molecular associations and reactions.
  Example: β_1-Adrenoreceptor interacts with a stimulatory G_S-protein to generated cAMP, whereas α_2-adrenoreceptor interacts with an inhibitory G_I-protein to attenuate cAMP production.
- Differences in receptor microenvironment and function at heterologous effector cells.
  Example: Nicotinic receptors at neuromuscular junction and ganglion cells.
- Differences in receptor microenvironment of homologous effector cells.
  Example: nAChR subtypes in various homologous effector cells.
- Differences in subcellular localization associated with functional diversity.
  Example: $GABA_B$ presynaptic autoreceptors and $GABA_A$ postsynaptic receptors.
- Differences in neuronal receptor sensitivity to neurotransmitter.
  Example: Cl^- gated channels of $GABA_A$ receptors containing $\beta_2\gamma_2$ subunits, such as the cerebral cortex, are more responsive to GABA than cells with $\beta 1\gamma 1$ subunits, which are more prevalent in the cerebellum.[5]
- Heterology of regulatory allosteric sites of receptors.
  Example: $GABA_A$ receptors in cerebellar pyramidal and granular cells comprising $\gamma 2$ subunits are sensitive to benzodiazepines, whereas cells containing $\alpha 2$ subunits are insensitive.[6]
- Differences in coupling efficiency of receptors to G-proteins.
  Example: A mutation of the $\beta 2$-adrenoreceptor favors its interaction with G_S, resulting in elevated production of cAMP.[7] Spontaneous mutation of G-protein-

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coupled receptors may result in various pathologies, as exemplified in the rhodopsin system where a point mutation in lys296, which restricts the attachment to retina, leads to increased sensitivity to light and, eventually, retinal degeneration.[8]

- Heteromeric subunit composition imparts differences in ion selectivity and/or conductance.
  Example: The AMPA-glutamate receptor subtype exhibits fast conductance, low Ca^{2+} permeability, and Mg^{2+} blockade; whereas the NMDA receptor exhibits slow conductance, high Ca^{2+} permeability, and Mg^{2+} insensitivity.[9] This distinction is attributed to a single amino acid in the M2 membrane spanning segment of the receptor subunit: a glu or arg in the AMPA receptor subunit (GluRB) and an asn in the NMDA subunit (NR1 or NR2).
- Receptor subtypes capable of coupling to different G-proteins.
  Example: A chicken m4 gene expressed in CHO cells exhibits forskolin-stimulated cAMP production and agonist-stimulated phosphoinositide turnover, whereas its expression in Y1 cells results in agonist-inhibited cAMP production, but no stimulation of phosphoinositide turnover.[10]
- Changes in receptor subunits during development associated with neuronal maturation.
  Example: In the adult nervous system activation of the glutamate-AMPA receptor elicits a current with a fast and steady-state component, whereas in the immature brain the desensitizing component is greatly reduced or absent.[11]
- The distribution of receptor subtypes may be a mechanism for targeting receptors to distinct neurons or neuroanatomic regions.
  Example: Type I sodium channels are located in pyramidal and dentate gyrus cell bodies, whereas type II channels are localized in myelinated fibers.[12]
- Variation of coupling efficiency of receptor subtype to G-proteins.
  Example: The sevenfold greater D1 receptor-stimulated cAMP production in the corpus striatum as compared to the substantia nigra appears to be related to the greater concentration of G_{olf} present in the striatum.[13] Because low levels of G_S are found in the substantia nigra, it would appear that the difference is due to distinct D1 receptor subtypes.
- Variations in allosteric sites of receptor subtypes regulating excitatory parameters.
  Example: The nAChRs exhibit multiple conformational states associated with activation, channel openings, and desensitization, involving sites that are distinct from the ACh recognition site.[14] Included among the physiologic and pharmacologic factors acting on such sites are divalent cations, neuropeptides, hormones, diverse neurotropic drugs, membrane potential, and phosphorylation.
- Phylogenetic changes in receptors.
  Example: Neurotransmitters and neuropeptides are present in primitive organisms lacking nervous systems, where they may be linked to signal transduction mechanisms via specific receptors regulating such factors as growth, differentiation, and cell motility.[15] Receptors and their subtypes underwent further development and diversification as neural control of such functions evolved.
- Variations in subunit composition of receptor subtypes associated with differences in conductance characteristics.
  Example: Studies with transfected cells expressing $\alpha1\beta2$, $\alpha1\gamma2$, and $\alpha1\beta2\gamma2$ subunits of rat $GABA_A$ receptors showed differences in the time characteristics of chloride channel conductance, depending primarily on the presence or absence of $\beta2$ and $\gamma2$ subunits. GABA-activated currents in cells expressing $\beta2$

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subunits exhibited faster desensitization, greater outward rectification, and shorter mean opening time than receptors composed of $\alpha 1\gamma 2$ subunits.[15]

OVERVIEW

The intent of this conference entitled Functional Diversity of Interacting Receptors was to contribute to a better understanding of the functional significance of receptor diversity, by addressing such topics as the genetic regulation and expression of receptor subtypes; the differential functional characteristics of neurons with distinct subtypes; second messenger and ion channel diversity associated with receptor isoforms; species and regional neuroanatomic differences in receptor subtypes; receptor changes associated with chronic exposure to agonists and antagonists and occurring with age and pathologic states; and the interaction of different receptors in functional regulation. Inasmuch as our knowledge of the structural, genetic, and functional features of the nicotinic cholinergic receptors appears to be more advanced than that of other receptors, the emphasis of the conference was on cholinergic receptors and their interaction with other receptors. Also included were presentations dealing with adrenergic, dopaminergic, histaminergic, gabaminergic, excitatory amino acid, and peptidergic receptors and their interactions and functional implications in health and disease.

The conference began with a presentation reviewing recent findings on the nature of ion channels associated with the various neuronal nAChR subtypes and describing the use of specific ligands for probing ion channels and nicotine's binding site. A discussion of the structure-activity relationships of novel subtype-selective opioid peptides was presented to illustrate the principles involved in the design of agonists and antagonists. With the use of synthetic peptides related to the α subunit of the nAChR, it was shown that α-bungarotoxin binds to amino acids in the 180–200 region (*Torpedo* numbering) of the α subunit, whereas κ-bungarotoxin binds to region 51–70 of the neuronal α_3 subunit. Two-dimensional [^{1}H]NMR studies on complexes formed between α-bungarotoxin and 18-mer peptide, corresponding to 185–196 sequence of the *Torpedo* α subunit, demonstrated the involvement of H186, W187, Y189, and Y190 in the contact zone. Patch clamp studies on cultured rat hippocampal neurons revealed the presence of two nAChR receptors with different properties: an α-bungarotoxin–sensitive one, which was shown by *in situ* hybridization to contain an α_7 subunit, and $\alpha_4\beta_2$.

Presynaptic nAChRs were shown to be involved in the release of norepinephrine (NE) from the CA1, CA3, and DG regions of the hippocampus, in the modulation of NE release from vas deferens and in the release of acetylcholine from the neuromuscular junction. The hippocampal nAChR was believed to comprise the $\alpha_3\beta_2$ subunits. With the use of photoreactive agonists for nicotinic cholinergic and other neurotransmitter receptors, a novel laser-pulse photolysis method with a microsecond time resolution was used to determine the rate constants for ion channel opening and closing, the concentration of the open channel, and the binding constants of inhibitors to sites on both the open and closed channels. The combined use of immunochemical, histochemical, and autoradiographic techniques was described for mapping the muscarinic cholinergic projections from the nucleus basalis to the thalamus and cerebral cortex, from the prepeduncular pontine nucleus to the thalamus, and other projections involving the sensory-limbic and reticular activating systems. By co-transfecting a cAMP response element-driven reporter gene with various muscarinic receptor genes, it was demonstrated that both the m_1 and m_4

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increase cAMP production, whereas high levels of expression of m_4 cause agonist-independent inhibition of adenylate cyclase via $G_{i\alpha 2}$.

Monoclonal antibodies to the various α and β subunits of nAChR were used to demonstrate that ganglia and retina contain uncertain combinations of α_5, β_2, and β_3 subunits, and brain, mainly the stoichiometry $(\alpha_4)2(\beta_2)3$ as well as two functional α-bungarotoxin-binding subunits, α_7 and α_8. A study of patterns of regulation of nAChR subtypes in various transfected cell lines revealed that ganglionic nAChR mRNA and function are stimulated by nerve growth factor, whereas nicotine exposure increased muscle nAChR numbers but with a loss of function. A presentation on brain nicotinic receptors and cholinergic transmission raised issues concerning (1) the relationship of cholinergic innervation to brain nicotinic cholinergic receptors as determined by such techniques as autoradiography, immunocytochemistry, and lesioning brain pathways; and (2) the functional significance of brain nicotinic receptors.

The afternoon session of the second day was devoted to the functional interaction of various neurotransmitter and neuropeptidergic receptors, describing their neuroanatomic distribution, subtype specificity, and their pathologic-therapeutic implications. Despite the fact that all three β-adrenergic receptors were structurally similar and bound to the same trimeric G_S-protein coupled positively to adenylate cyclase, they exhibited striking differences in their agonist-antagonist profile and regulation, which were attributed to specific point mutations. A number of presentations referred to the role of antipsychotic drugs in elevating brain neurotensin in the neostriatum. On the basis of findings demonstrating that haloperidol both decreased and increased neurotensin mRNA—the former via D_3 and the latter via D_2 receptors in the nucleus accumbens—it was inferred that the antipsychotic drugs alleviated positive symptoms (e.g., hallucinations, anxiety) by acting on D_2 receptors, whereas negative symptoms (e.g., stereopoty, increased self-stimulation) appear to involve D_3 receptors. Neuroanatomic combined with receptor binding studies demonstrated interactions between dopamine receptors and cholecystokinin 8, which produces behavioral effects (sedation, catalepsy, antistereopoty) opposite to those of dopamine. Localization of the peptide binding domain of the substance P receptor was performed by attachment of a photolabile amino acid, *p*-benzoyl-L-phenylalanine, to positions 4 and 8 of substance P and structural analysis of the photolabeled substance P receptor fragments isolated from receptor-transfected CHO cells.

Renewed interest in the functional role of histamine in the central nervous system has resulted from the cloning and expression of the H_1, H_2, and H_3 receptors. The H_1 receptor increased cAMP, inositol phosphates, and arachidonic acid, whereas the H_2 receptor increased cAMP and decreased arachidonic acid production. 1-Methylhistamine, which has a 1500-fold greater affinity than histamine for the H_3 receptor, may be an endogenous candidate for the neuronal H_3 autoreceptor.

The presentations on opioid receptors dealt with topics ranging from the purification of a μ-receptor, cloning and expression of κ-receptors, structure-activity relationships of subtype-selective opioid peptides, to receptor changes after chronic exposure to opiate agonists and antagonists. With the cloning of the various opioid receptor subtypes the techniques of recombinant expression, immunocytochemistry, and *in situ* hybridization have been used to eludicate the functional as well as subcellular and neuroanatomic localization of subtypes of opioid receptors in brain and spinal cord. Among the novel findings were that δ-receptor is found mainly on axons, the μ-receptor on plasma membranes of axons, cell bodies, and dendrite, and that the enkephalin-containing terminals are proximal to δ- or μ-receptors.

Molecular genetic studies of the melanocortin (MSH) receptor revealed it to be a unique bifunctionally controlled receptor, positively regulated by MSH, resulting in

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brown-black pigmentation, and negatively regulated by the agouti peptide, resulting in variable pigmentation in coat color.

The final session dealt with the structural, neuroanatomic, functional, and clinical aspects of the various excitatory amino acid (EAA) and GABA receptors. To date three subtypes of EAA receptors are known: a quisqulate- or AMPA-sensitive one existing in four isoforms with 70% homology, a kainate-sensitive one with 75% homology, and a NMDA-sensitive one existing in five isoforms with 25% homology. Studies with selective agonists and antagonists suggest that the NMDA receptor is implicated in brain damage associated with status epilepticus, the quisqulate receptor with general anesthesia, and the NMDA receptor with head injury. The release of GABA from cultured retinal cells was mediated by glutamate receptors and found to be calcium-dependent and blocked by nifendipine. Bilateral lesioning of connections between the rat temporal cortex and the lateral entorhinal cortex resulted in an impairment of retroactive and proactive memory and a reduction in glutamate receptors and loss of terminals in both regions. In addition to its involvement in the release of intracellular Ca^{2+}, EAA receptor-mediated formation of inositol phosphates (IP) stimulates Ca^{2+} entry in synaptosomes via a voltage-gated channel, leading to a further increase in IP.

Following the demonstration that the memory deficit in rats was attenuated by cycloserine, a glycine-receptor agonist, clinical trials were undertaken in Alzheimer patients with encouraging results. NMDA receptors appear to be involved in the negative symptoms associated with schizophrenia that are not responsive to antidopaminergic neuroleptics, but appear, in preliminary clinical trials, to be attenuated by large doses of glycine. The hypothesis that nicotine interacts with the mesolimbic dopaminergic system to improve working memory gained additional support from studies demonstrating that the administration of dopamine agonists along with nicotine improved performance of rats in a radial maze.

Investigations of the subunit composition of the $GABA_A$ receptor by immunocytochemical and *in situ* hybridization techniques revealed the existence of multiple forms in all brain areas with the major subunit composition $\alpha_1 \beta_2 \gamma_2$. Bergmann glia cells of the cerebellum were found to express γ_1 and α_2. A monoclonal antibody for the $GABA_B$ receptor was shown to inhibit the binding of agonists to the receptor and to prevent the GABA-mediated inhibition of adenylate cyclase in cerebral synaptic membrane preparations.

In order to account for the shift in the control of hepatic glycogenolysis from α_{1B} to the inhibitory β_2-adrenergic receptors following hepatic injury or malignancies, a mechanism was proposed involving the translocation of protein kinase C (PKC) from the cytoplasm to the plasma membrane. In addition to PKC, which is involved in the uncoupling of the α_{1B}-receptor and coupling of β_2-receptor, arachidonic acid appears to play a role in the conversion.

The poster sessions included such topics as the modulations of the m_2 muscarinic receptor by nitric oxide; autoregulation of ACh release in rat cerebrum by both a stimulatory m_5 and inhibitory m_1 muscarinic receptor subtype; α_2-adrenergic regulation of body temperature via inhibition of warm-sensitive and inhibition of cold-sensitive hypothalamic neurons and by interaction with serotonergic and dopaminergic systems; interference by peptide YY of cholecystokinin's inhibition of pancreatic secretion; antagonism by polyamines of imipramine-induced immobility in rats; inability to account for the hypotensive action of methionine enkephalin by changes in tyrosine hydroxlase and catecholamine metabolizing enzymes; advantages of combined agonist-antagonist administration in smoking cessation therapy; detection of the Tax gene in human macrophages infected with HTLV-1; expression and functional characterization of an 140–204 amino acid fragment of the α subunit of

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nAChR; and determination of glutamate receptor subtypes involved in calcium influx and the modulation of dopamine release in hippocampal synaptosomes.

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